CLAIMS

What is claimed is:

1. A gene silencing site-specific recombination system comprising:

a) a first recombinase element having the general structure P1-R, wherein P1 is a first promoter and R is a recombinase coding sequence and 3' region; and

b) a second gene silencing-recombinase element having the general structure: RS-X-RS*-Y

wherein:

i) RS and RS* are opposingly oriented recombinase sites responsive to the recombinase;

ii) X is a nucleic acid fragment comprising at least one second promoter in a 3' to 5' orientation; and

iii) Y is a nucleic acid fragment comprising at least one target sequence;

wherein expression of the recombinase results in inversion of the element contained between RS and RS* and transcription of the second gene silencing-recombinase element resulting in the production of double-stranded RNA and silencing of the target gene.

2. A gene silencing site-specific recombination system according to claim 1 wherein the target sequence of Y is in a 3' to 5' orientation.

3. A gene silencing site-specific recombination system according to claim 1 wherein X is selected from the group consisting of: $P2_{INV}$, TS_{INV}- $P2_{INV}$, and 5' Intron-TS_{INV}- $P2_{INV}$, and wherein Y is selected from the group consisting of TS-$P3_{INV}$, TS_{INV} –polyA, 3' Intron-TS_{INV} - polyA, wherein:

a) $P2_{INV}$ and $P3_{INV}$ are inverted second and third promoters whose orientation is from 3'-5';

b) TS is a target sequence;

c) TS_{INV} is an inverted target sequence whose orientation is from 3'-5';

d) polyA is the 3' region of a gene;

e) 5' Intron is the N-terminal portion of an intron; and

f) 3' Intron is the C-terminal portion of an intron.

4. A gene silencing site-specific recombination system comprising:

a) a first recombinase element having the general structure P1-R, wherein P1 is a first promoter and R is a recombinase coding sequence and 3' region; and

b) a second gene silencing-recombinase element having the general structure: P2-TS-RS-Z-RS-TS_{INV}-polyA;

wherein:

a) P2 is a second promoter;

b) TS is a target sequence;

c) RS is a recombinase site responsive to the recombinase

d) Z is a nucleic acid fragment selected from the group consisting of a STOP fragment and an intron;

e) TS_{INV} is an inverted target sequence whose orientation is from 3'-5'; and

f) polyA is the 3' region of a gene.

5. A gene silencing site-specific recombination system according to any of Claims 1 or 4 wherein the recombinase and recombinase site are selected from the group consisting of *Cre*-lox, FLP/FRT, R/RS, Gin/gix, a pSR1 system, a cer system, and a fim system.

6. A gene silencing site-specific recombination system according to any of Claims 1 or 4 wherein the first promoter and at least one second promoter are selected from the group consisting of

a) constitutive plant promoters;

b) plant tissue-specific promoters;

c) plant development stage-specific promoters;

d) chemically-inducible plant promoters; and

e) viral promoters.

7. A gene silencing site-specific recombination system comprising:

a) a first recombinase element having the general structure P1-R, wherein P1 is a first promoter and R is a recombinase coding sequence and 3' region; and

b) a second gene silencing-recombinase element having the general structure: RS-TS_{INV}- $P2_{INV}$-RS*- TS_{INV} -polyA, wherein:

i) RS and RS* are opposingly oriented recombinase sites responsive to the recombinase;

ii) TS_{INV} is an inverted target sequence whose orientation is from 3'-5';

iii) $P2_{INV}$ is an inverted second promoter whose orientation is from 3'-5'; and

iv) polyA is the 3' region of a gene;

wherein P1 and P2 are operably linked to their down stream elements and wherein expression of the recombinase results in inversion of the element contained between RS and RS* and transcription of the gene silencing-recombinase element resulting in production of double-stranded RNA and silencing of the target gene.

8. A gene silencing site-specific recombination system comprising:

a) a first recombinase element having the general structure P1-R, wherein P1 is a first promoter and R is a recombinase coding sequence and 3' region; and

b) a second gene silencing-recombinase element having the general structure RS-5' Intron-TS_{INV}- $P2_{INV}$-RS*-3' Intron-TS_{INV} - polyA, wherein:

i) RS and RS* are opposingly oriented recombinase sites responsive to the recombinase;

ii) 5' Intron is the N-terminal portion of an intron;

iii) TS_{INV} is an inverted target sequence and whose orientation is from 3'-5';

iv) $P2_{INV}$ is an inverted second promoter whose orientation is from 3'-5';

v) 3' Intron is the C-terminal portion of an intron; and

vi) polyA is the 3' region of a gene;

wherein P1 and P2 are operably linked to their down stream elements and wherein expression of the recombinase results in inversion of the element contained between RS and RS* and transcription of the gene silencing-recombinase element, resulting in excision of the intron by mRNA splicing and production of double-stranded RNA and silencing of the target gene.

9. A gene silencing site-specific recombination system comprising:

a) A recombinase element having the general structure P1-R, wherein P1 is a first promoter and R is a recombinase coding sequence and 3' region; and

b) A gene silencing-recombinase element having the general structure RS-$P2_{INV}$-RS*-TS-$P3_{INV}$, wherein:

i) RS and RS* are opposingly oriented recombinase sites responsive to the recombinase;

ii) $P2_{INV}$ and $P3_{INV}$ are inverted second and third promoters respectively, whose orientation is from 3'-5'; and

iii) TS is a target sequence;

wherein P1, P2, and P3 are operably linked to their down stream elements and wherein expression of the recombinase results in inversion of the element contained between RS and RS* and transcription of the gene silencing-recombinase element resulting in production of double-stranded RNA silencing the target gene.

10. A gene silencing site-specific recombination system according to Claim 9 wherein the target sequence has a poly A region operably-linked at its 3' end.

11. A gene silencing site-specific recombination system comprising:

a) A recombinase element having the general structure P1-R, wherein P1 is a first promoter and R is a recombinase coding sequence and 3' region; and

b) A gene silencing-recombinase element having the general structure P2-TS-RS-STP-RS-TS_{INV}-polyA, wherein:

i) P2 is a second promoter;

ii) TS is a target sequence;

iii) RS is a recombinase site responsive to the recombinase;

iv) STP is a Transcriptional 'STOP' fragment;

v) TS_{INV} is an inverted target sequence and whose orientation is from 3'-5'; and

vi) polyA is the 3' region of a gene;

wherein P1 and P2 are operably linked to their down stream elements and wherein expression of the recombinase results in excision of the element contained between RS and transcription of the gene silencing-recombinase element resulting in production of double-stranded RNA silencing the target gene.

12. A gene silencing site-specific recombination system comprising:

a) A recombinase element having the general structure P1-R, wherein P1 is a first promoter and R is a recombinase coding sequence and 3' region; and

b) A gene silencing-recombinase element having the general structure P2-TS-5' Intron-RS-STP-RS-3' Intron-TS_{INV}-polyA, wherein:

i) P2 is a second promoter;

ii) TS is a target sequence;

iii) 5'Intron is the N-terminal portion of an intron;

iv) RS is a recombinase site responsive to the recombinase;

v) STP is a Transcriptional 'STOP' fragment;

vi) 3'Intron is the C-terminal portion of an intron;

vii) TS_{INV} is an inverted target sequence whose orientation is from 3'-5'; and

viii) polyA is the 3' region of a gene;

wherein P1 and P2 are operably linked to their down stream elements and wherein expression of the recombinase results in excision of the element contained between RS and transcription of the gene silencing- recombinase element resulting in excision of the intron by mRNA splicing and production of double-stranded RNA silencing the target gene.

13. A gene silencing site-specific recombination system according to any one of Claims 8-12 wherein the first promoter is a germline promoter.

14. A gene silencing site-specific recombination system according to Claim 14 wherein the germline promoter is selected from the group consisting of:

a) constitutive plant promoters;

b) plant tissue-specific promoters;
c) plant developmental stage-specific promoters;
d) chemically-inducible plant promoters;
e) viral promoters;
f) male germline-specific promoters;
g) female germline-specific promoters;
h) common germline-specific promoters;
i) floral common germline-specific promoters;
j) vegetative shoot apical meristem-specific promoters; and
k) floral shoot apical meristem-specific promoters.

15. A gene silencing site-specific recombination system according to Claim 14 wherein the male germline-specific promoter is derived from genes selected from the group consisting of genes specific to anther primordia, anther sporophyte and pollen gametophyte.

16. A gene silencing site-specific recombination system according to Claim 14 wherein the common germline-specific promoter is derived from genes selected from the group consisting of *Apetala* 3 (AP3), *Pistillata* (PI), synthetic anther promoter, TA29, BCP1 and orthologs thereof.

17. A gene silencing site-specific recombination system according to Claim 16 wherein the common germline-specific promoter is derived from genes selected from the group consisting vegetative and floral shoot apical meristems.

18. A gene silencing site-specific recombination system according to Claim 18 wherein the common germline-specific promoter is derived from genes selected from the group consisting of *Leafy* (LFY), *Apetala* 3 (AP3), *Pistillata* (PI), *Apetala* 1 (AP1), *Agamous* (AG), *Pistillata* (PI) and orthologs thereof.

19. A gene silencing site-specific recombination system according to Claim 14 wherein the floral common germline-specific promoter is derived from genes selected from the group consisting of

Agamous (AG), *Apetala* 1 (AP1), *Apetala* 3 (AP3), *Leafy* (LFY) and orthologs thereof.

20. A gene silencing site-specific recombination system according to Claim 14 wherein the vegetative shoot apical meristem-specific promoter is selected from the group consisting of *Agamous* (AG), *Apetala* 1 (AP1), *Apetala* 3 (AP3), *Leafy* (LFY), *Aintegumenta* (ANT), *Clavata* 3 (CLV3), *Wushel* (WUS), *Meristemless* (STM) and orthologs thereof.

21. A gene silencing site-specific recombination system according to any of Claims 7-9 and 11-12 wherein the second promoter and optionally, third promoter, are selected from the group consisting of:

a) constitutive plant promoters;
b) plant tissue-specific promoters;
c) plant development stage-specific promoters;
d) chemically-inducible plant promoters; and
e) viral promoters.

22. A gene silencing site-specific recombination system according to any of Claims 1, 4 and 7-9 and 11-12 wherein the target sequence silences a target gene selected from the group consisting of:

a) a gene encoding an enzyme of a biosynthetic pathway;
b) a gene encoding a storage protein;
c) a gene conveying sterility;
d) a gene conveying a specific phenotype on a plant or plant cell;
e) a hormone biosynthetic gene; and
f) a gene involved in gene silencing.

23. A gene silencing site-specific recombination system according to Claim 22 wherein the genes involved in gene silencing are selected from the group consisting of:

a) *qde-1*, *qde-2*, and *qde-3;*
b) *rde-1*, *rde-2*, *rde-3*, and *rde-4*;
c) *mut-2*, and *mut-7*;
d) *ego-1;*
e) *AGO1*;

f) *SGS-2/SDE-1*, *SGS-1*, and *SGS-3;*

g) RdRP;

h) Dicer; and

i) homologs of thereof.

24. A gene silencing site-specific recombination system according to any of Claims 7-9 and 11-12 wherein the recombinase coding sequences and recombinase site are selected from the group consisting of *Cre*-lox, FLP/FRT, R/RS, Gin/gix, a pSR1 system, a cer system, and a fim system

25. A gene silencing site-specific recombination system according to any of Claims 7-9 and 11-12 wherein the recombinase element and the gene silencing-recombinase element may be genetically linked or unlinked.

26. A gene silencing site-specific recombination system according to Claim 25 wherein the recombinase element and the gene silencing-recombinase element may be genetically unlinked and reside in different plants.

27. A method for silencing a target gene, comprising introducing into a plant cell comprising a target gene, a gene silencing construct of any one of Claims 1, 4, 7-9 and 11-12 wherein expression of the recombinase results in translation of the target sequence and the production of double-stranded target sequence RNA.

28. A method according to Claim 27 wherein P1, P2, $P2_{INV}$, and optionally P3, are selected from the group consisting of:

a) constitutive plant promoters;

b) plant tissue-specific promoters;

c) plant development stage-specific promoters;

d) chemically-inducible promoters; and

e) viral promoters.

29. A method according to Claim 28 wherein P1 is selected from the group consisting of:

a) male germline-specific promoters;

b) female germline-specific promoters;
c) common germline-specific promoters;
d) flower-specific promoters;
e) vegetative shoot apical meristem-specific promoters; and
f) floral shoot apical meristem-specific promoters.

30. A method according to Claim 29 wherein P1 is an inducible promoter responsive to heat shock.

31. A method according to Claim 29 wherein P1 is an inducible promoter responsive to a chemical safener.

32. A method according to Claim 29 wherein P1 is a floral SAM or flower promoter.

33. A method according to Claim 27 wherein the recombinase element and the gene silencing-recombinase element are genetically unlinked and reside in different plants.

34. A method for expressing conditional gene silencing in a plant comprising:

A) providing constructs comprising:

i) a first recombinase element having the general structure P1-R;

ii) a second gene silencing-recombinase element having a general structure selected from the group consisting of:

1) RS-$P2_{INV}$-RS-TS-$P3_{INV}$;

2) RS-TS_{INV}- $P2_{INV}$-RS- TS_{INV}–polyA;

3) RS-5' Intron-TS_{INV}- $P2_{INV}$-RS-3' Intron-TS_{INV} – polyA;

4) P2-TS-RS-STP-RS-TS_{INV}-polyA; or

5) P2-TS-5' Intron-RS-STP-RS-3' Intron-TS_{INV}-polyA;

wherein:

a) P1 is a first promoter;

b) R is a recombinase;

c) RS is a recombinase site responsive to the recombinase;

d) P2 and P3 are second and third promoters that have overlapping expression profiles and are either the same or different;

e) $P2_{INV}$ is an inverted second promoter whose orientation is from 3'-5';

f) TS is a target sequence optionally having an operably-linked poly A region at the 3' end of each complementary strand;

g) TS_{INV} is an inverted target sequence whose orientation is from 3'-5';

h) 5'Intron is the N-terminal portion of an intron;

i) 3'Intron is the C-terminal portion of an intron;

j) STP is a Transcriptional 'STOP' fragment; and

k) polyA is the 3' region of a gene;

wherein P1, P2, $P2_{INV}$, and optionally P3, are operably linked to their down stream elements;

B) providing a first plant having a target gene and comprising the first recombinase element;

C) providing a second plant having a target gene and comprising the second gene silencing- recombinase element; and

D) crossing the first and second plants to produce progeny in which expression of the recombinase under the control of P1 promoter inverts or excises the elements between the recombinase sites on the second gene silencing-recombinase element permitting the formation of double-stranded RNA encoding the target sequence to silence the target gene in the plant.

35. A method for effecting systemic gene silencing in a plant comprising:

A) providing constructs comprising:

i) a first recombinase element having the general structure P1-R;

ii) a second gene silencing-recombinase element having a general structure selected from the group consisting

1) $RS\text{-}P2_{INV}\text{-}RS\text{-}TS\text{-}P3_{INV}$;

2) $RS\text{-}TS_{INV}\text{-}P2_{INV}\text{-}RS\text{-}TS_{INV}$–polyA;

3) RS-5' Intron-TS_{INV}- $P2_{INV}$-RS-3' Intron-TS_{INV} – polyA;

4) P2-TS-RS-STP-RS-TS_{INV}-polyA; or

5) P2-TS-5' Intron-RS-STP-RS-3' Intron-TS_{INV}-polyA;

iii) a third gene-silencing-mobility element having the general structure P4-TS_R;

wherein:

a) P1 is a first promoter that is chemically inducible;

b) R is a recombinase;

c) RS is a recombinase site responsive to the recombinase;

d) P2 and P3 are second and third promoters that have overlapping expression profiles and are either the same or different;

e) $P2_{INV}$ is an inverted second promoter whose orientation is from 3'-5';

f) TS is a target sequence optionally having an operably-linked poly A region at the 3' end of each complementary strand;

g) TS_{INV} is an inverted target sequence whose orientation is from 3'-5';

h) 5'Intron is the N-terminal portion of an intron;

i) 3'Intron is the C-terminal portion of an intron;

j) STP is a Transcriptional 'STOP' fragment; and

k) polyA is the 3' region of a gene;

l) P4 is a strong constitutive promoter; and

m) TS_R is redundant target sequence comprising at least a target sequence selected from the group consisting of TS and TS_{INV};

wherein P1, P2, $P2_{INV}$, optionally P3, and P4 are operably linked to their down stream elements;

B) providing a plant having a target gene and comprising the first recombinase element, the second gene silencing-recombinase element, and the third gene silencing-mobility element; and

C) chemically inducing the P1 promoter to cause inversion or excision of the elements between the recombinase sites on the second gene silencing-recombinase element permitting the formation of double-stranded RNA encoding the target sequence to

silence the target gene and creation of a mobile gene-silencing signal in the plant for systemic silencing.

36. A method according to claim 35, wherein TS_R is a repressor of a trait gene.

37. A method according to claim 35, wherein the plant comprising the first recombinase element, the second gene silencing-recombinase element, and the third gene silencing-mobility element is the product of a genetic cross.

38. A method according to claim 35, wherein the plant comprising the first recombinase element, the second gene silencing-recombinase element, and the third gene silencing-mobility element is the product of co-transformation.